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THE PREPARATION OF BLOOD SMEARS AND TISSUE IMPRESSIONS FOR LABORATORY STUDY

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Satisfactory examination of any pathological specimen is largely dependent upon its proper preparation. This is especially true in examinations for blood parasites of animal forms. Poorly prepared specimens not only delay the work of the examiner but often are entirely worthless. They result in a needless loss not only of the specimens but also of time and energy on the part of the examiner in the search for good fields for microscopic study. The loss of materials in many cases is of critical importance. It is for the purpose of reducing the waste of time and materials that the following suggestions and illustrations have been prepared for fieldmen of the Fish and Wildlife Service and others cooperating.

Two types of preparations and preparation methods are described. While the end result in each type is practically the same, the method of procedure, as will be shown, is of necessity different.

The two kinds of preparations here dealt with are blood smears and tissue impressions. For either type, a standard 1-by-3-inch clear-glass microscope slide is preferred. Lack of such a slide, however, need not be a deterrent if valuable or rare specimen material must be attended to in the absence of preferred facilities, since window glass of approximately this size and shape or irregular pieces that may be shaped later may be used.

PREPARATION OF BLOOD SMEARS

For satisfactory microscopic examination, fixed blood smears should consist of thin films, one layer of cells deep, on clean, clear-glass slides. These can be made with blood freshly drawn from the animal, in which case they must be prepared immediately before clotting takes place, or they may be made at a later time from a blood sample that, to prevent clotting, has been citrated at the time of collection. Two-tenths of a cubic centimeter of 2-percent sodium citrate in normal saline solution is commonly used for each cubic centimeter of blood. The citrate solution can usually be procured at local drug stores. Whether fresh or citrated blood is used, the procedure in preparing a smear is much the same.

Blood in small quantities is most easily and safely obtained from live birds from the radial or tarsal veins. Puncture of the radial vein is easily accomplished with a fine sharp needle on the under side of the wing where the vein crosses obliquely on the proximal side of the humero-radial joint. In small mammals the veins of the ear or tail are most commonly tapped. If quantities of 1/2 to 1 cc. or more are desired, heart puncture with a hypodermic needle and syringe is most satisfactory, but this should be employed only by trained persons, since in the hands of the novice many fatalities result.

As the blood wells to the surface, a small drop can be picked up on the corner of a clean slide (fig. 1, A, slide 1) and placed in the center, laterally, of a second clean slide (fig. 1, A, slide 2) about half an inch from one end (fig. 1, B). The clean end of slide No. 1 should then be placed as indicated in the illustration with the drop of blood in the acute angle formed between the slides where it will immediately spread across both of them (fig. 1, C). With a steady, one motion sweep, slide No. 1 still held at the same angle is propelled the remaining length of slide No. 2 (fig. 1, D). As the slide is pushed forward, a thin, even film of blood is left behind.

Care should be taken so to regulate the size of drop that the film will not be heavy enough to make an opaque smear. The ideal size results in a smear that feather-edges out about a quarter of an inch from the end of the slide. The wet smear should be allowed to air-dry in a box where it will be protected from dust and out of contact with any object. Unstained dry blood smears are avidly attacked by flies, cockroaches, crickets, and silverfishes, and will soon be destroyed.

Freezing the blood film before drying results in hemolysis of the cells, which ruins the slide. If it is necessary to work in subfreezing temperature, provision must be made to keep the blood films warm until they dry. This applies also to tissue impressions and to tissues from which impressions are to be made.

PREPARATION OF TISSUE IMPRESSIONS

When carcasses are available but no fluid blood, certain tissues may be found to carry small quantities of free blood cells. The best material for providing them is lung tissue, a small cube of which, about half a centimeter on a side, is snipped free with scissors. The freshly cut surfaces are then applied by a series of light-pressure contacts to one side of a clean slide (fig. 1, E). No overlapping of the contact spots should be permitted. Four to six or eight regularly placed impressions per slide are satisfactory. If the tissue is extremely dry so that no blood stain is visible on the slide, or if watery and the impressions are clear, the preparation will probably be of little value. When too much liquid interferes with making good impressions, part of the excess fluid may be taken up by making a few contacts on clean paper toweling or filter paper before impressions are made on the slide.

When impressions of liver and spleen tissues are desirable, these should be prepared in the same manner as the lung tissue. Cells of the liver and spleen are often needed in addition to any contained blood, so fresh-cut surfaces should be used.

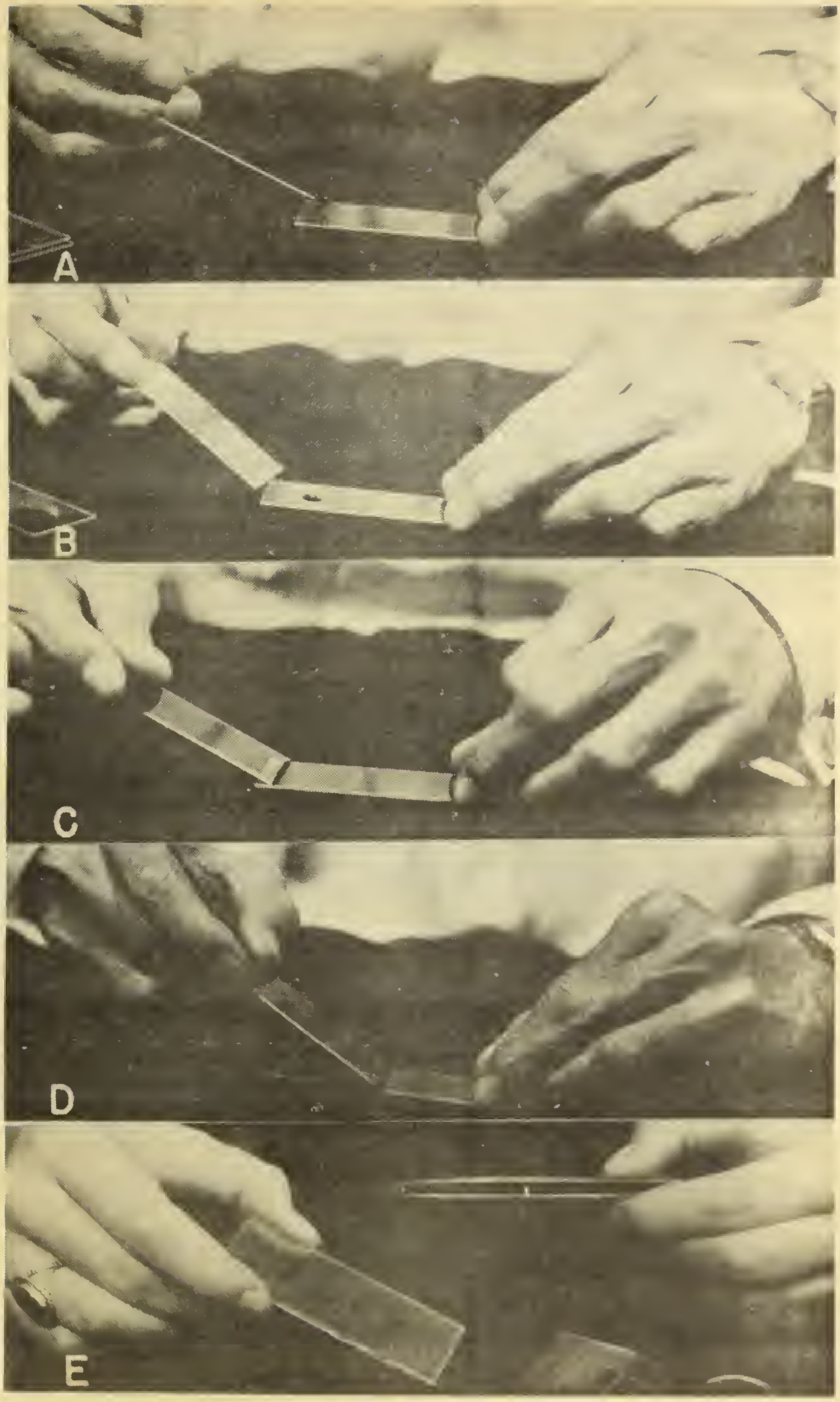

FIGURE 1.—A, Slide and drop of blood ready to be placed on it; B, drop in place ready for spreading with clean end of first slide; C, drop has been touched and has spread across within the acute angle formed by the slides; D, spreading the blood smear; E, preparation of tissue-contact impressions. (BS320M)